Experimental Synthesis and Characterization of EK(NDI) and Computational Insights to the UV-vis Absorbance Behavior of EK(NDI) and EFEK(DAC): Studies Towards the Integration of Experiment and Computation for Peptide Conjugates

Research Thesis

Presented in Partial Fulfillment of the Requirements for graduation *with research distinction* in Chemistry in the undergraduate colleges of The Ohio State University

by

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Abstract

Peptide conjugates are one of many approaches to creating functional nanomaterials for biological, photo-chemical, and other applications. The use of chromophores in these peptide conjugates is ubiquitous as it both adds a means of assembly for the conjugate and also a marker to investigate the conjugates experimentally using UV-vis absorption and circular dichroism spectroscopies. The use of these spectroscopies is prevalent in the biochemical research field, and the circular dichroism technique is powerful in terms of its ability to relay structural information. In the world of peptide conjugates, this might also prove to be a powerful tool for revealing structural information to the experimentalist. In order to understand the interaction of a molecule with circularly polarized light, an understanding of the UV-vis properties of a molecule must be attained. This research is divided into two parts. First, the peptide conjugate EK(NDI) was synthesized and characterized by multiple forms of spectroscopy including UV-vis absorbance. One primary finding is that a monomer unit could not correctly reproduce the UV characteristics of the assembly in water. Second, the molecule EFEK(DAC) was investigated. This molecule's UV-vis characteristics were modeled under one pH condition and agree with experimental UV-vis spectra. The other pH conditions, where the molecule is assembled, however, could not be modeled with one molecule. This is a direct result of the abilities of multiple chromophores to interact in the assembled state of the peptide conjugate, which is under current investigation.

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I would like to thank my organic lecture professor and research advisor, Dr. Jon R. Parquette. His view that organic chemistry can be like art inspired me and has impacted how I have done research. His mentoring throughout both my research and studies has been greatly appreciated. In addition, his kindness in accepting me into his group and allowing me the freedom to synthesize a new molecule has been formative in my research views.

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1. Introduction

1.1 Background: The Design of Chromophore Peptide Conjugates

Supramolecular chemistry is one of today's fastest growing fields. Governing this field is a new set of rules that inspire the synthetic strategy of molecular self-assembly. These self-assembly methods have been influenced by biology as well as chemical intuition, and all methods are contingent on the ability to control intermolecular interactions.[1] Controlling these intermolecular interactions allows for the formation of nano-aggregates, which may be functionalized as photo-electronic devices, drug carriers, and biomaterials.[2] In the bulk of published work, these systems contain one self-assembling molecule either covalently or non-covalently interacting with a functional non-self-assembling molecule (or macromolecule).[3] However, the ability to use more than one self-assembling material in a segregated co-assembly presents an opportunity that would allow for more complex devices to be made. In the Parquette group, these devices have been investigated in recent years through the design of chromophore peptide conjugates. These conjugates function with a design of alternating polar and non-polar amino acids, which create an amphiphilic molecule with a preferential growth direction.[2b]

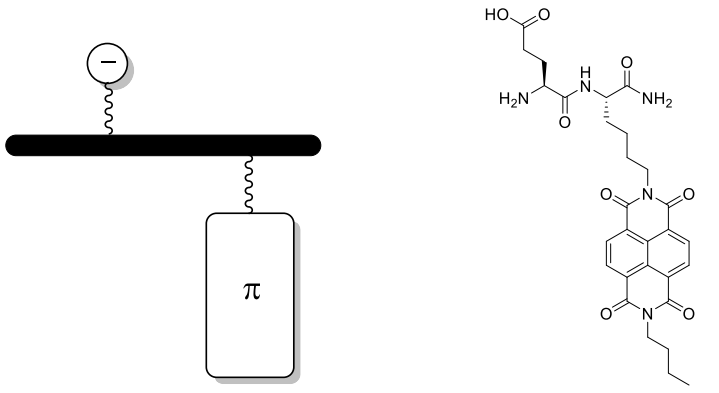

Figure 1.1. A figure which shows the alternating amino acid sequence in a dipeptide conjugate system beside a peptide conjugate. The middle segment of the cartoon represents the carbon backbone, and on either side are the polar and non-polar amino acids.

1.2 Avenue of Investigation

In the lab's ongoing research, peptide conjugates have been designed with the goal of creating a discrete co-assembled nanostructure. The current methods being investigated have used charge complementarity between the polar residues of the peptide conjugates being co-assembled. Preliminary results have shown promise in creating co-assembled structures; however, a greater understanding of the structure of the individual assemblies may prove to be useful in the design of new structures for co-assembly. In this project, two peptide conjugates, EFEK(DAC) and EK(NDI), were investigated by the use of experimental UV-vis absorbance spectroscopies and computational TDDFT methodologies. In addition, the compound EK(NDI) was synthesized by the author, so the characterization and synthesis of this molecule is described within. The goal of simulating the absorbance spectra of these compounds is two-fold. First, computing the absorbance spectra of monomers lays the foundation for calculations using a greater number of molecules. Second, the UV-vis characteristics of EFEK(DAC) present an interesting case in which one peak splits into two upon self-assembly, which is a rare phenomenon.[4]

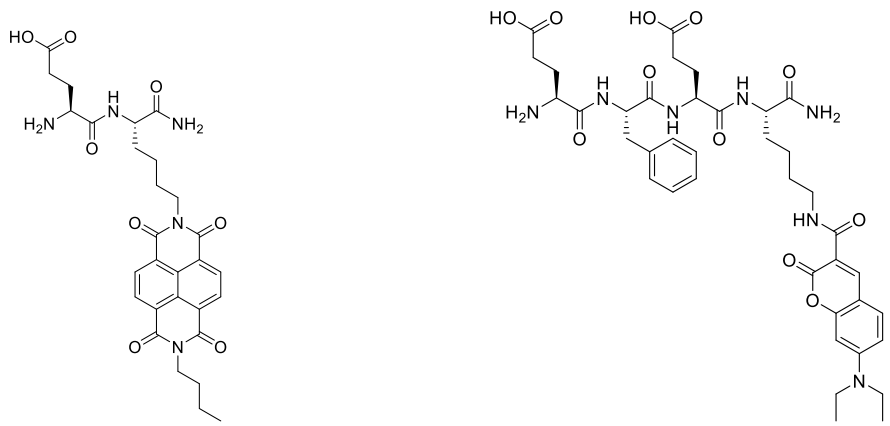

Figure 1.2. Shown above are the two structures under investigation within at their proposed protonation states in neutral water, EK(NDI) left and EFEK(DAC) right.

1.3 Naming of Oligo-peptide Conjugates

The naming system for peptide conjugates used within this paper is as follows. The amino acids are listed from the N to C terminus. The prefix to the amino acids one letter abbreviation, or lack thereof, indicates what the N-terminus is covalently linked to the prefix, or if left blank, indicates a free amine. The C-terminus is left blank for all conjugates discussed within and is assumed to be an amide. Lastly, the use of parentheses, typically after lysine, is means that that molecule is covalently conjugated to the amino acid before it. Figure 1.3 shows the directionality of naming, and the amide functional group at the C-terminus of the strand and the lysine linkage may be seen in Figure 1.2**.**

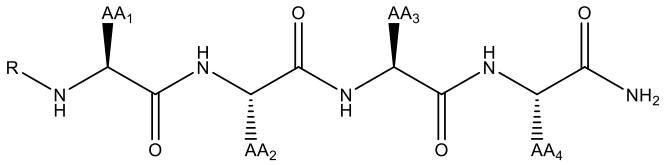

Figure 1.3. In the figure, a tetrapeptide template is given for naming conventions.

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2. The Synthesis and Characterization of EK(NDI)

2.1 Synthesis of EK(NDI)

The synthesis of the EK(NDI) peptide comprised three steps: the synthesis of the naphthalene diimide (NDI) chromophore, the solid-phase synthesis of the peptide backbone, and the coupling of the chromophore to the backbone. The resulting crude peptide was then cleaved from the resin with acid, evaporated under vacuum, and then precipitated with diethyl ether. The crude peptide was then dried under nitrogen and purified by reverse phase HPLC. The product was found to elute in a large band centered at 17.9 minutes using a programmed gradient of 20% to 100% acetonitrile in water in 30 minutes. The eluent containing the product was then frozen and lyophilized. The compound was then confirmed by mass spectrometry with a [M] of 579.23, found [M+1] of 580.28. Full procedures and synthetic schemes are provided in the appendix.

2.2 Characterization of the EK(NDI) Supramolecular Polymer

The conditions for the self-assembly of the EK(NDI) supramolecular polymer were to take the lyophilized product and quantitatively transfer it by dissolving the powder in membrane filtered water (Nanopure) and transferring the resulting solution to a weighed glass vial, refreezing it and lyophilizing again to a powder. Once dry, the powder was re-dissolved in Nanopure water at the initial concentration, 10 mM, before being diluted to the concentration required by the experiment and aged for two weeks. By transmission electron microscopy (TEM), the resulting structure was found to have a fiber-like morphology with an average fiber diameter of 16.5 nm. The fibers were observed to have grooves, which is indicative of multiple ribbon-type bilayers stacking to form a mature fiber as seen in Figure 2.1. At lower concentrations and at the ends of larger fibers, ribbons were observed.

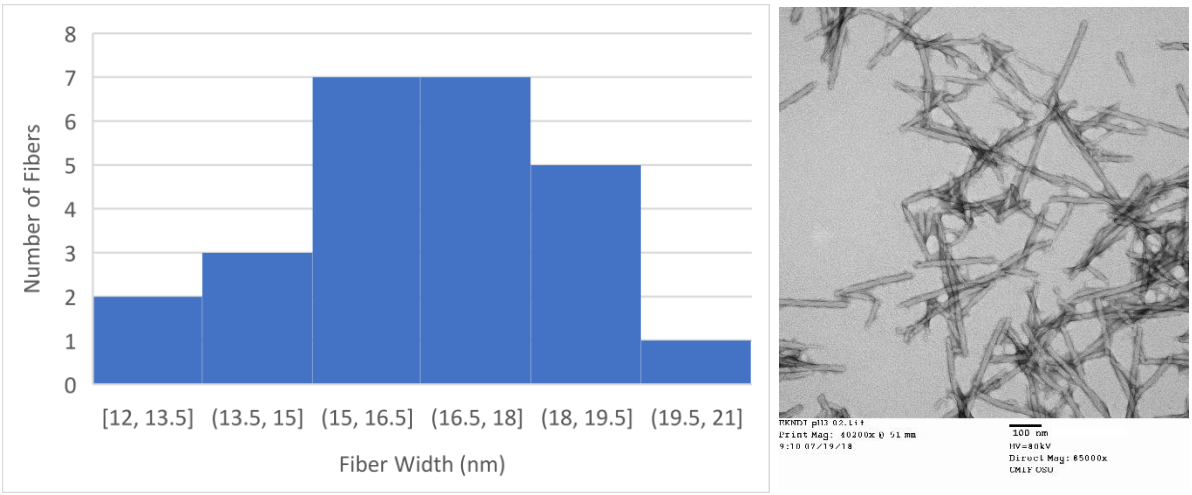

Figure 2.1. On the left is a histogram of fiber diameters as seen by TEM. The sample was prepared for TEM by taking EK(NDI) aged at 10 mM for 2 weeks, and diluting to 1 mM immediately before application to the grid. The resulting solution was applied to a carbon coated copper grid for one minute and the excess was removed. The grid was then stained with 2% (w/w) uranyl acetate solution for 30 seconds and the excess was then removed.

The UV-vis absorbance characteristics of the assembly were investigated by varying concentration of EK(NDI) in both water and 2,2,2-trifluoroethanol (TFE). In water, the assembly exhibits a large peak centered around 390 nm, which red-shifted with increasing concentration. Assuming the fraction of assembled molecules increased with concentration, this bathochromic shift would be indicative of J-type aggregation.[1] In TFE, a large peak was observed at 381 nm. In contrast to the assembly in water, the peak did not shift with concentration. This difference was examined by plotting the absorption dependence for the two systems at 381 nm as a function of concentration. In the case of the TFE dissolved monomer, the wavelength dependence was linear, indicating only one species was present. In the case of the water-dissolved monomer, the same curve was non-linear, which is indicative of multiple states (monomeric and assembled) in the solution. These two facts together confirm the J-type aggregation in this peptide conjugate when assembled in water.

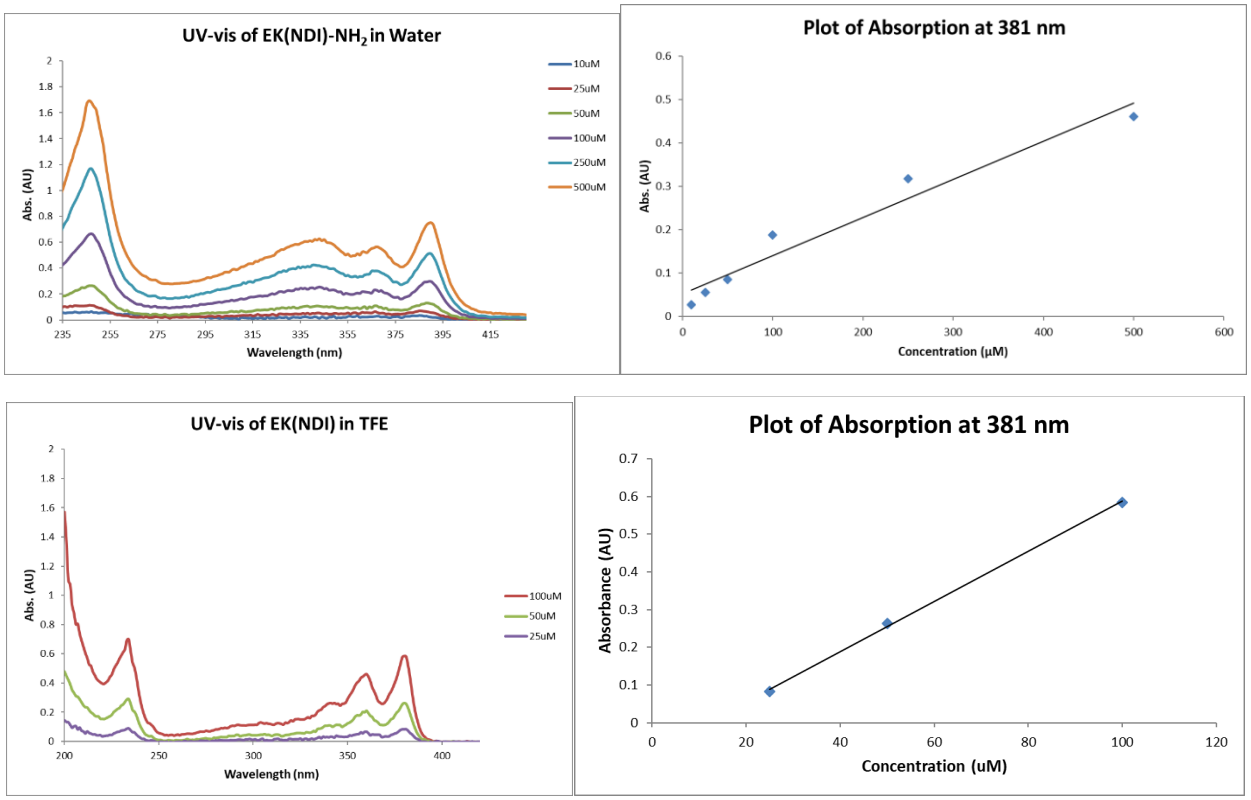

Figure 2.2. Pictured on the left are the absorbance spectra of the EK(NDI) peptide conjugate in water, top, and in TFE, bottom. To the right of the absorbance graphs are the plots of absorption at 381 nm for the respective solvation. All measurements were made by first preparing a stock solution of EK(NDI) at 10 mM in either Nanopure water or TFE. The stock was then diluted to the concentrations displayed in the graphs UV-vis analysis.

After characterization of the absorbance, the assembly was observed by circular dichroism spectroscopy (CD). The CD spectra of the assembly exhibited an exitonic couplet in the 245 nm region as well as multiple overlaid exitonic couplets in the 340-390 nm range. Consistent with the the absorption experiments, it was found in the CD that as concentration increased, the exitonic splitting of the peak increased. Similar to the UV-vis data the coupling energy increased with concentration, which indicated that more chromophores were integrated into the assembly as the concentration increased.

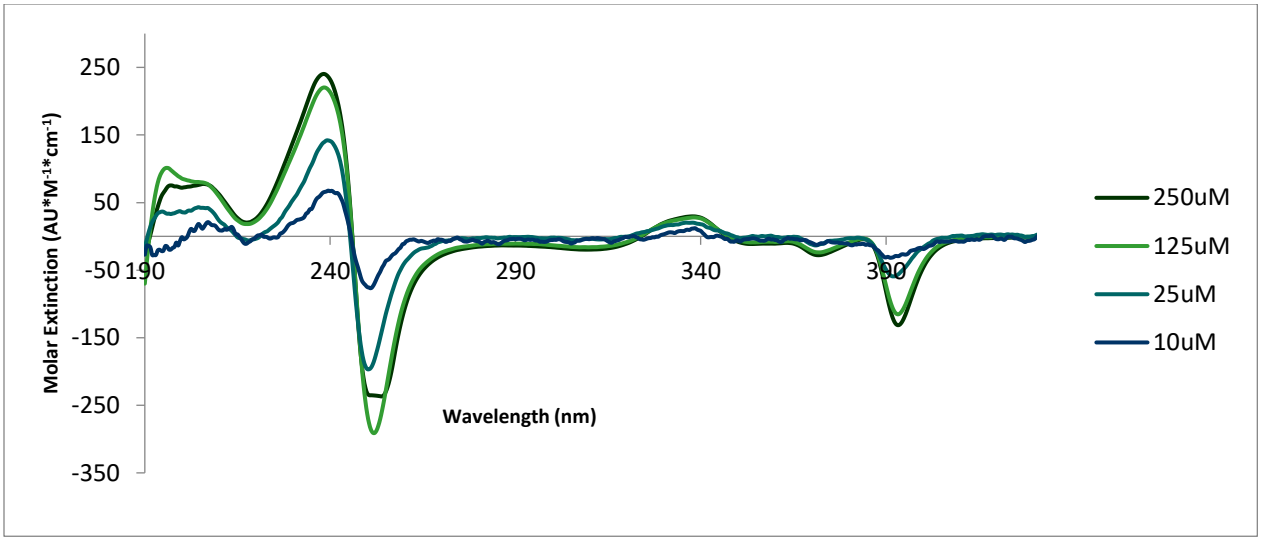

Figure 2.3. Graph showing the CD spectra of various concentrations of EK(NDI) with units of molar extinction. The samples were prepared for CD by first making a stock solution of EK(NDI) at 10 mM in membrane-filtered water. The experimental solutions were then prepared by dilution of this stock solution with additional water.

Additional testing in various pH conditions was completed in order to investigate the stability of the assembly. For these experiments, the assembly was first prepared at the 10 mM concentration as for the UV-vis experiment. The pH of the solution was then adjusted with NaOH or HCl to four pH conditions (3, 5, 7, and 9) and then let age. Upon aging samples for two weeks, no morphological changes were seen by TEM. The lack of change was further confirmed through CD spectroscopy which showed near identical curves for each sample as seen in Figure 2.4. This indicated that from pH 3-9 the protonation state of the peptide conjugate remained constant and therefore the assembly did not change, or that the pre-assembled structure was resistant to morphological change triggered by pH between 3 and 9.

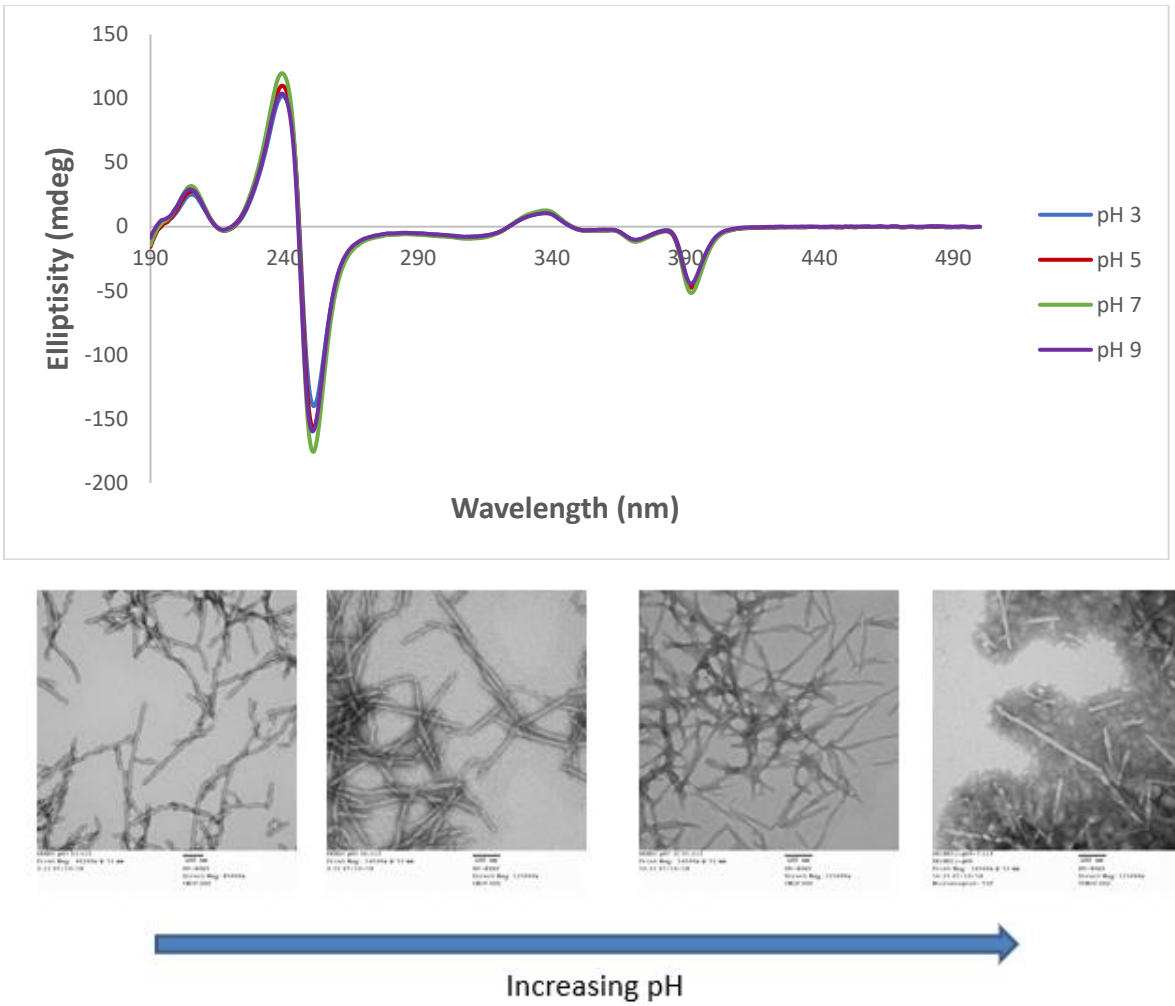

Figure 2.4. At the top is the CD graph for EK(NDI) under the four pH conditions. Below, is a figure showing the lack of morphological change as pH is increased. The samples were prepared for TEM by taking EK(NDI), aged at 10 mM for 2 weeks at the desired pH, and diluting to 1 mM immediately before application to the grid. The resulting solutions were applied to carbon coated copper grids for one minute and the excess solutions were removed. The grids were then stained with 2% (w/w) uranyl acetate solution for 30 seconds and the excess was then removed.

The last feature characterized was the XRD pattern observed from the assembly. The sample was prepared by drop casting a 11 mM solution of EK(NDI) onto a silicon wafer. The sample was irradiated using a Cu Kα1 source, and the sample was scanned from 5° to 35° in a 2Θ orientation. The resulting data was plotted in the d-space by using Braggs law to convert from 2Θ to distance. Two weak peaks were observed corresponding to 4.75 Å and 6.3 Å. The

first peak corresponded to be the inter-sheet spacing of a beta-sheet arrangement of the peptide subunits of silk fibers, while the other was proposed to be the spacing of monomers perpendicular to the strand's direction of growth.[2] This indicates that the fibrillar aggregates assemble via a beta-sheet motif, which may be used to assemble starting structures in computational simulations for dimers and other larger systems.

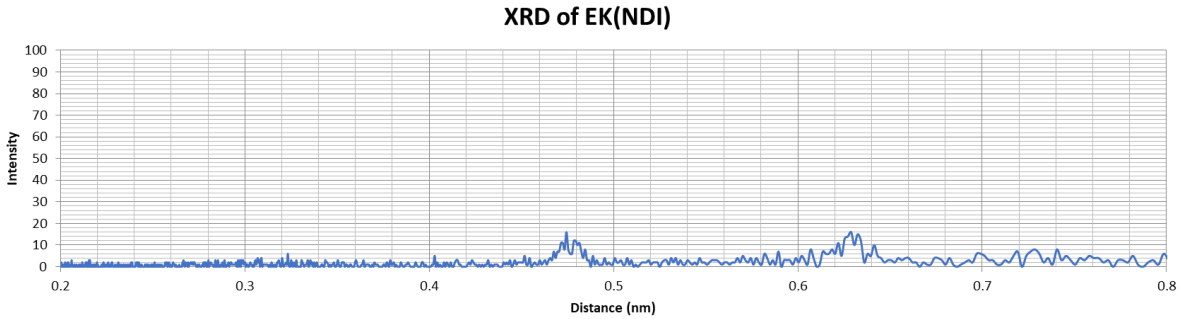

Figure 2.5. The XRD plot is shown above in d-space for the drop-cast EK(NDI) thin film. The thin film was made by drop casting 11 mM EK(NDI) to a silicon wafer. The drops were dried under nitrogen and the silicon wafer was adjusted to set into a Bruker D8 Advance X-Ray Powder Diffractometer sample holder.

2.3 References

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3. Computational Modeling of Two Peptide Conjugates

3.1 Computational Modeling of EK(NDI)

In order to investigate the observed UV-vis characteristics of the assembled and disassembled structure, simulations for the UV-vis spectra of both a truncated chromophore and the full peptide conjugate were done. Specifically, the truncated chromophore was built as a n-butyl amide (as if the chromophore were chopped from the lysine alpha carbon). The resulting structure, referred to as dibutyl NDI, was first built in Spartan and a conformational search was done using the MMFF98 force field.[1] From the top ten conformers, the top two were analyzed as they were almost identical in energy and were 5 kJ/mol lower in energy than any of the other conformers.

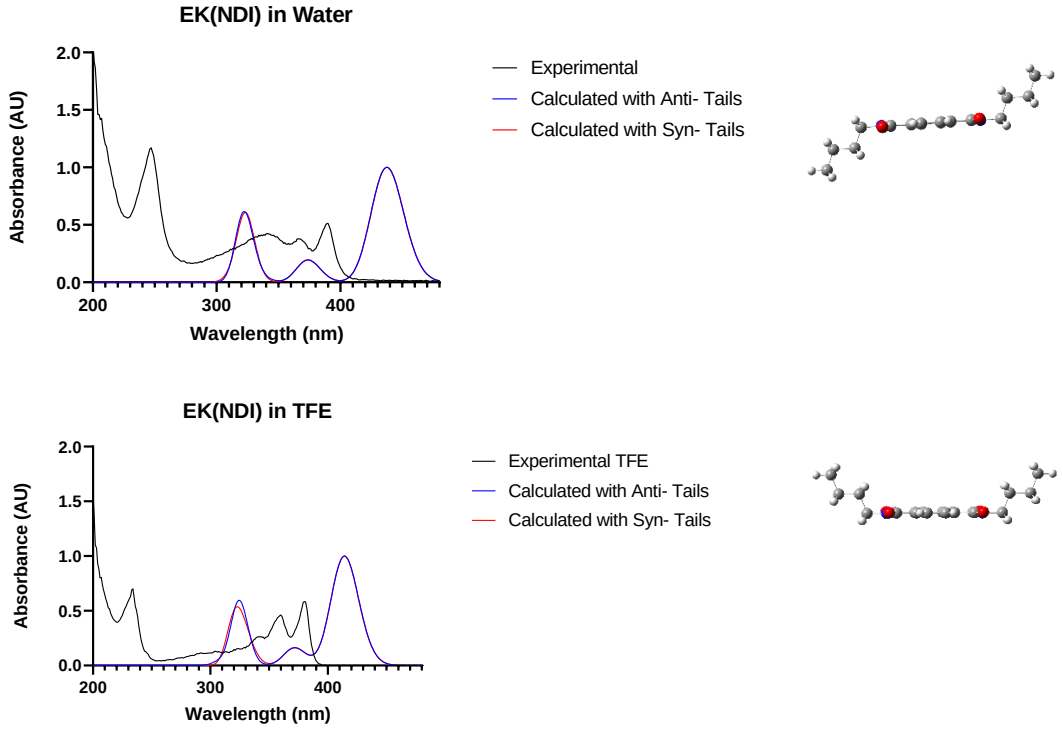


Figure 3.1. Shown on the left are the two absorbance graphs of EK(NDI) in water, top, and TFE, bottom. Shown on the right are the two conformers of dibutyl NDI used, anti on top and syn on bottom.

This compound was not synthesized in the lab. However, it was used in order to assess the smallest structure that could be representative of the peptide conjugate computationally. The two conformers were transferred to Gaussian and geometry optimized with the B3LYP method and the 6-31G+* basis set. From the optimized geometry, electronic transitions were calculated with the same method and basis set.[2] Then, UV-vis graphs, orbitals, and difference density plots were extracted for the excitations of interest using the GaussView6 software.[3]

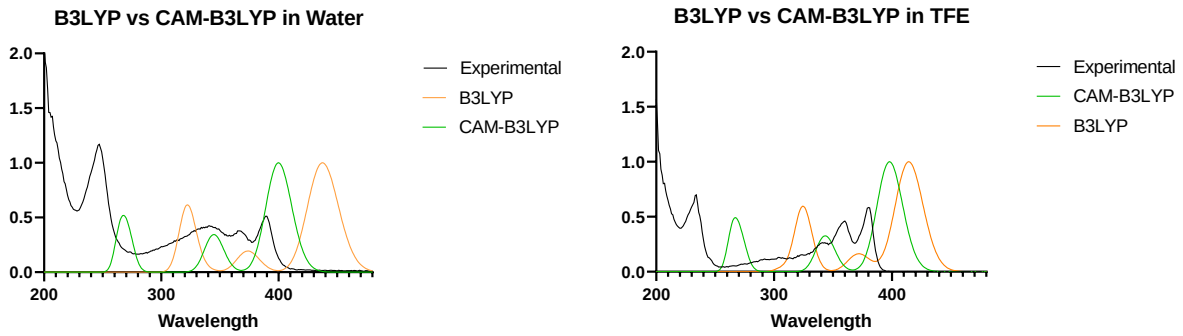


Figure 3.2 Shown are plots of calculated absorption spectra for the dibutyl NDI with anti tails. On the left is a water solvation model compared to the absorbance spectra of EK(NDI) in water, and on the right is a TFE solvation model compared to the absorbance spectra of EK(NDI) in TFE.

After these initial calculations, it was determined that the B3LYP method overestimated the experimental energies of the transitions, as well as failing to capture the shape of the curve. Consequently, CAM-B3LYP was investigated as another method, which, due to its correction of longer range effects, might better calculate the energy of the transition. The same geometry was used and the electronic transitions were calculated using this new method. This method was successful in that it approximated a lower wavelength transition. Despite the shift to a more similar wavelength, the shape of the curve was not correct, so it was determined that the conjugate could not be modeled by the chromophore alone. This was seen in the number of excitations in the 300 nm to 400 nm region, which did not match the experimental data. This

indicated that the NDI amide could not model peptide conjugate in water or TFE, and that the CAM-B3LYP functional better approximates the energies of the transitions.

The peptide conjugate was then put through the same building and conformational searching processes as the dibutyl NDI structure. From the conformational search, the top conformer was taken and the geometry was optimized according to the same procedure, and the same electronic structure was calculated using both the B3LYP and CAM-B3LYP methodologies.

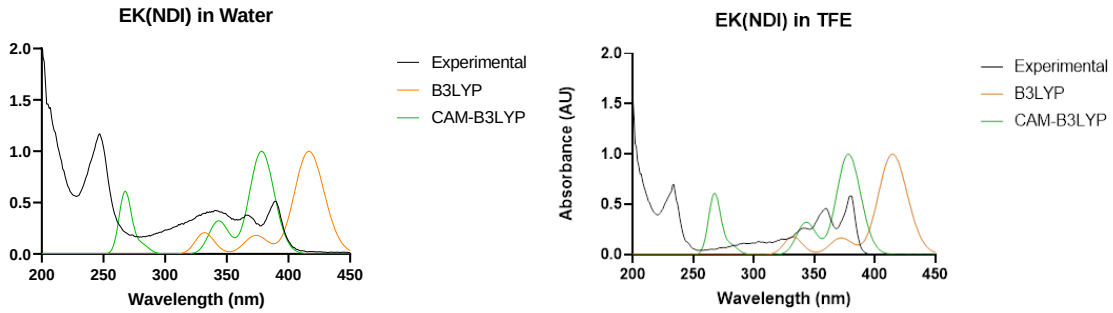


Figure 3.3 Pictured above are the simulated UV-vis absorption spectra for the full EK(NDI) conjugate. On the left is the system with a water solvation model and on the right is the system with a TFE solvation model.

From these calculations it was determined that the shape absorption spectra of the EK(NDI) conjugate was not correctly reproduced. This indicated that the three transitions seen in the experimental spectrum must be a result of coupling between adjacent chromophores in solution. In addition, CAM-B3LYP calculated a more accurate energy of the first peak than B3LYP, supporting that it better models the NDI chromophore.

3.2 Computational Modelling of EFEK(DAC)

The peptide conjugate EFEK(DAC) is a molecule of current investigation in the Parquette group due to its interesting self-assembly, which undergo changes in morphology triggered by pH. Specifically, the conjugate forms nanotubes at pH 4, sheet-like structures at pH 7, and disassembles at pH 10. When investigating this molecule, it was noticed that upon assembly at pH 4 and 9, the single peak present in monomeric, pH 10, UV-vis spectra split into two overlapping peaks of equal intensity. In order to understand the electronic spectra from the bottom up, the DAC chromophore was first investigated aside from the full peptide conjugate. Previous work within the group allowed for comparison to experimental data for the DAC n-butyl amide at two pH conditions: 10 and 4. Therefore, the chromophore was modeled in both protonated and deprotonated forms at the amine position.

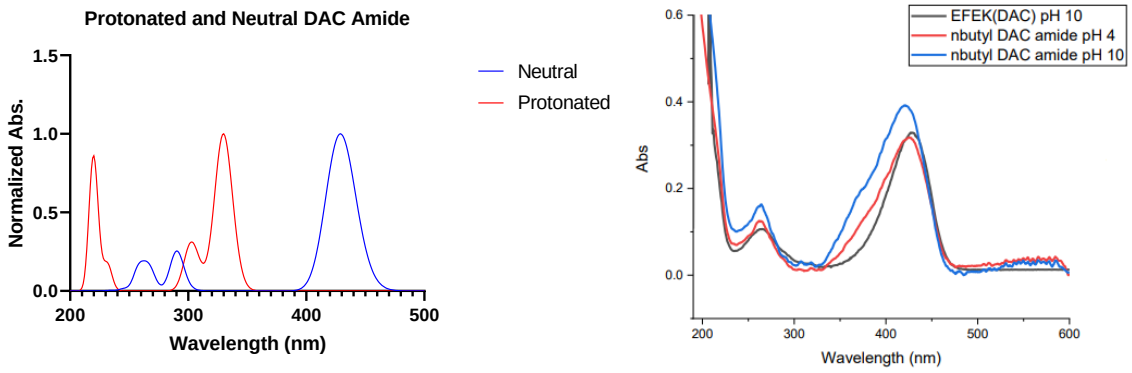


Figure 3.4. On the left is the calculated absorbance spectra of the protonated and neutral DAC amide molecules. On the right is the experimental absorbance of the DAC amide at pH 4 and 10.

The protonated and deprotonated molecules were built in the Spartan GUI and a conformational search was done using the MMFF98 force field to determine the minimum energy conformers. The top conformation from both searches was taken, transferred to a Gaussian file, and geometry optimized using the B3LYP method and the 6-31G+* basis set, with solvation from the SMD model for water in Gaussian 16. From the optimized structures, the UV-vis was calculated in solution and molecular orbitals extracted.

Through this analysis, it was found that the energy of transition for the DAC n-butyl amide most closely resembled the deprotonated form at both experimental pH's. This indicated that the predominant protonation state of that nitrogen in the DAC amide was deprotonated in solution. From this insight, a series of protonation states for the peptide conjugate was made in order to model the structure of the full conjugate.

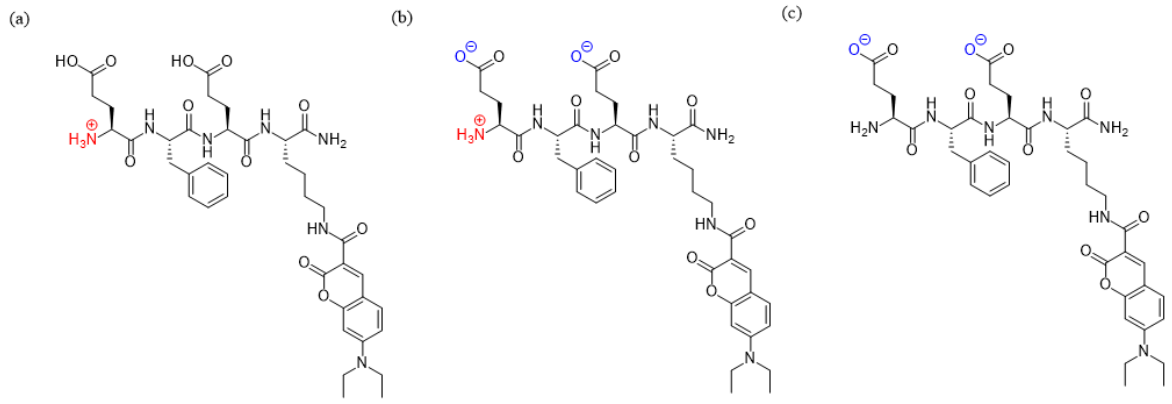


Figure 3.5. Pictured above are the proposed protonation states at the pH conditions which give rise to morphological changes. (a) pH 4. (b) pH 7. (c) pH 10.

The peptide conjugate was then investigated through calculations of the electronic transitions for the monomeric form of the EFEK(DAC) assembly. In this calculation, the proposed EFEK(DAC) monomer was built in Spartan and a conformational search was done. The lowest energy conformer was then transferred to Gaussian 16 and a geometry optimization was run in the gas phase using the B3LYP method with the 6-31G+* basis set. At the resulting geometry, the electronic transitions were calculated using the same method and basis set.

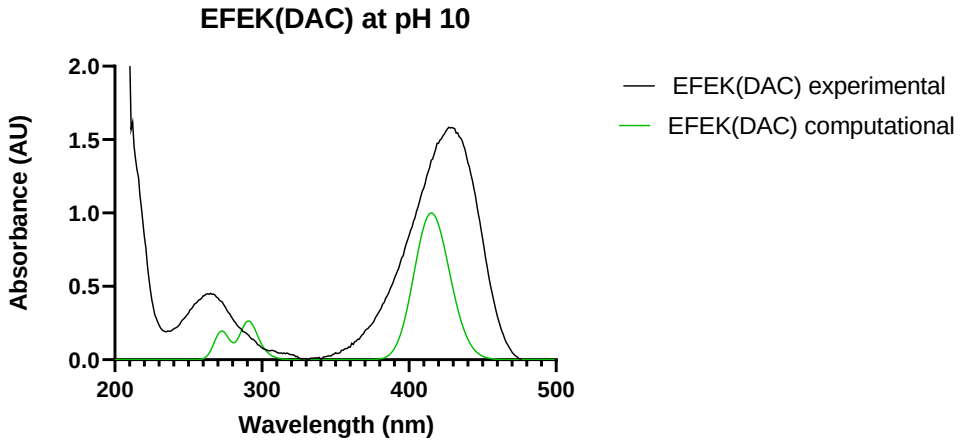


Figure 3.6. Above is a graph of the experimental and computational UV-vis absorbance spectra for the EFEK(DAC) peptide conjugate at pH 10 (monomeric conditions).

From this experiment, it was clear that the experimental spectrum was well approximated by the computed one. Specifically, both features were found to be within 10 nm of their experimental counterparts. The other protonation states of this molecule were not modeled here, as considerations for the assembly conditions were required. In all, the calculations for the DAC amide and EFEK(DAC) monomers showed that protonation of the diethyl amine was not likely in the assembled structure, and that B3LYP with the 6-31+G* basis set was adequate for modeling both structures.

3.3 References

1. Spartan'16 Wavefunction, Inc. Irvine, CAExcept for molecular mechanics and semi-empirical models, the calculation methods used in Spartan have been documented in: Y. Shao, L.F. Molnar, Y. Jung, J. Kussmann, C. Ochsenfeld, S.T. Brown, A.T.B. Gilbert, L.V. Slipchenko, S.V. Levchenko, D.P. O'Neill, R.A. DiStasio Jr., R.C. Lochan, T. Wang, G.J.O. Beran, N.A. Besley, J.M. Herbert, C.Y. Lin, T. Van Voorhis, S.H. Chien, A. Sodt, R.P. Steele, V.A. Rassolov, P.E. Maslen, P.P. Korambath, R.D. Adamson, B. Austin, J. Baker, E.F.C. Byrd, H. Dachsel, R.J. Doerksen, A. Dreuw, B.D. Dunietz, A.D. Dutoi, T.R. Furlani, S.R. Gwaltney, A. Heyden, S. Hirata, C-P. Hsu, G. Kedziora, R.Z. Khalliulin, P. Klunzinger, A.M. Lee, M.S. Lee, W.Z. Liang, I. Lotan, N. Nair, B. Peters, E.I. Proynov, P.A. Pieniazek, Y.M. Rhee, J. Ritchie, E. Rosta, C.D. Sherrill, A.C. Simmonett, J.E. Subotnik, H.L. Woodcock III, W. Zhang, A.T. Bell, A.K. Chakraborty, D.M. Chipman, F.J. Keil, A.Warshel, W.J. Hehre, H.F. Schaefer, J. Kong, A.I. Krylov, P.M.W. Gill and M. Head-Gordon, Phys. Chem. Chem. Phys., 8, 3172 (2006).

2. Gaussian 16, Revision A.03, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2016.

3. GaussView, Version 6, Roy Dennington, Todd A. Keith, and John M. Millam, Semichem Inc., Shawnee Mission, KS, 2016.

4. Conclusions and Current Work

4.1 Conclusions

In this research, the novel peptide conjugate EK(NDI) was presented and characterized. Then, in an effort to understand its assembly, the electronic transitions of the EK(NDI) subunit were modeled. It was found that using a truncated chromophore could not replicate the absorbance characteristics of the peptide conjugate in either monomeric or assembled form. The full conjugate, however, replicated the UV-vis shape. A second peptide conjugate, EFEK(DAC), was then investigated due to its peculiar UV-vis properties. The truncated chromophore was first modeled and compared to experimental data. It was found that in both pH regimes, acidic and basic, the DAC chromophore was deprotonated at the amine position. From there, the full conjugate was explored in three charge regimes in order to replicate the conditions at the three different pHs explored in the characterization of the conjugate. It was found that the monomer could be modeled, and that the model agreed with the experimental data.

4.2 Current Work

The current work of the project focuses on taking the acquired information from the electronic structure calculations for use in modeling the circular dichroism and absorbance of dimer systems. This presents a more complex problem than the modeling of single molecules due to the fact that conformational searching becomes impractical for multiple molecules. In order to tackle this problem, different beta-sheet alignments of the peptide conjugates have been optimized as possible structures. This has yielded initial results that reproduced the experimental absorbance-splitting phenomenon. In addition to this method, molecular mechanics is being investigated as a way to generate initial dimer concentrations. Once possible conformers are found, they will be put through the same treatment as the beta-sheet inspired conformations: optimizing geometry at a high level of theory and then carrying out electronic structure

calculations. If this does not give satisfactory results, molecular dynamics simulations may be used in which a dimer system is simulated to find the lowest energy conformation in each of the charge states. From here the same treatment will be given, and conformers will be investigated until experimental results are reproduced.

5. Experimental

General Methods. All Transmission Electron Microscopy was done using the Technai G2 Spirit at an operating voltage of 80kV. ^{1}H NMR was recorded at 400 MHz on a Bruker Avance III instrument. ESI-MS was carried out on a Bruker MicrOTOF coupled to HPLC. UV-Vis absorbance measurements were performed on a Shimadzu UV-vis using a 1 mm path length quartz cuvette at room temperature. Circular dichroism measurements were done using a JASCO J-815 CD Spectrometer using a 1 mm path length quartz cuvette at room temperature. For the pH study, a Mettler Toledo MP 125 pH meter was used with an InLab Micro pH probe. All water used in synthesis was filtered by a Millipore Direct-Q system.

Synthesis of *n*-Bu-NDI. *n*-Bu-NDI was synthesized according to previously published work (Ulijn et. Al. 2014). To a 1000 mL round bottom flask, 300 mL of nanopure water was added and degassed by bubbling nitrogen through the solution. To the flask naphthalene dianhydride (2.02 g, 7.5 mmol) was added with vigorous stirring. In four additions, KOH solution (35 mL, 1M) was added over the course of one minute. Once the NDA had fully dissolved, the solution was acidified using phosphoric acid (1M) to a pH around 6.4 as checked by pH paper. To the resulting solution, n-butyl amine (0.74 mL, 7.5 mmol) was added and the solution was re-acidified to a pH around 6.4. The reaction vessel was then flushed with nitrogen and the solution was refluxed for 18 h at 110°C. After refluxing, the solution was let cool to room temperature and then acidified to a pH of 1 with nitric acid (70%). The resulting precipitate was sedimented by centrifugation, and the supernatant was decanted. The wet precipitate was then frozen and lyophilized to yield a fluffy, beige powder.

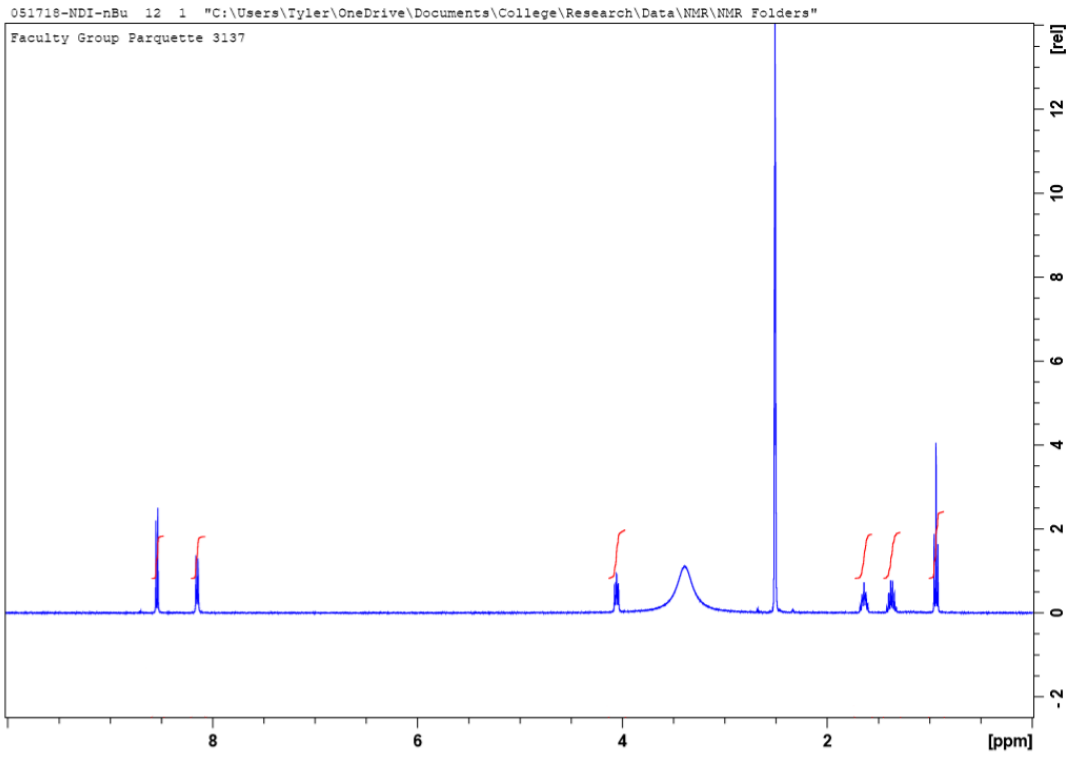


Figure E1. ^{1}H NMR for monobutyl NDI. Taken at 400MHz in DMSO.

Synthesis of EK(NDI). The dipeptide conjugate was prepared using Fmoc solid-phase peptide synthesis on rink amide resin (loading 0.3 mmol/g). The resin was first swelled with DCM for 2 h. Then, the first coupling step was done using Fmoc-amino acid, 1hydroxybenzotriazole (HOBt), and 1,3-diisopropylcarbodiimide (DIC) (3 equivalents each relative to the resin) in DMF for 2 h. For the addition of the second residue, Fmoc-amino acid, HOBt, DIC, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), and diisopropylamine (DIPEA) (3 equivalents each relative to resin) in DMF were reacted for 2 h. For Fmoc removal, a solution of 20% piperidine in DMF was used 3 times for five minutes each. For Mtt removal, 1% TFA in DCM was used, rinsing 7 times for 5 minutes and once with a drop of triethylsilane (TES) for five minutes. For the chromophore coupling step, HOBt, DIC, and n-butyl NDI (3 equivalents each relative to the resin) in DMF were reacted for 24 h.

Between coupling steps and after deprotections, a wash cycle of 3 rinses with DMF, 2 rinses with MeOH, and 2 rinses with DCM was used (each rinse lasting one minute). The final dipeptide conjugate was cleaved from the resin by treatment with TFA/TES/water (94/5/1) at room temperature for 1.2 h followed by one 5-minute rinse with cleaving solution. The cleaving solution was then evaporated under vacuum until no solvent appeared to distill off. From the resulting solution, the crude peptide was precipitated with diethyl ether and purified by reversed-phase HPLC on a preparative Varian Dynamax C18 column eluting with a linear gradient of CH_3CN/water containing 0.1% TFA (30%-100% CH_3CN over 30 minutes) and stored as lyophilized powders under nitrogen at 0°C. ;ESI-MS calcd for $C_{29}H_{33}N_5O_8$ $[M+H]^+$ 580.23, found 580.28.

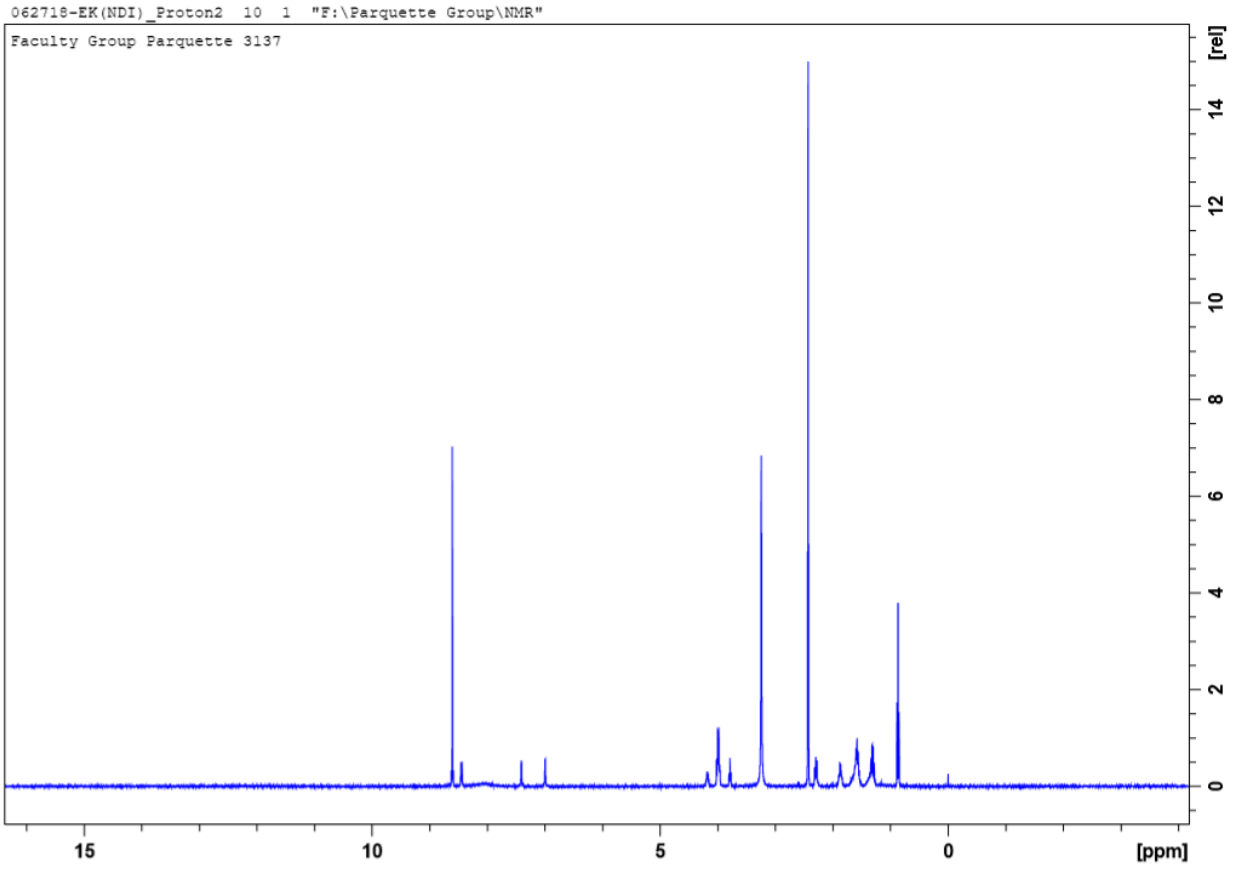
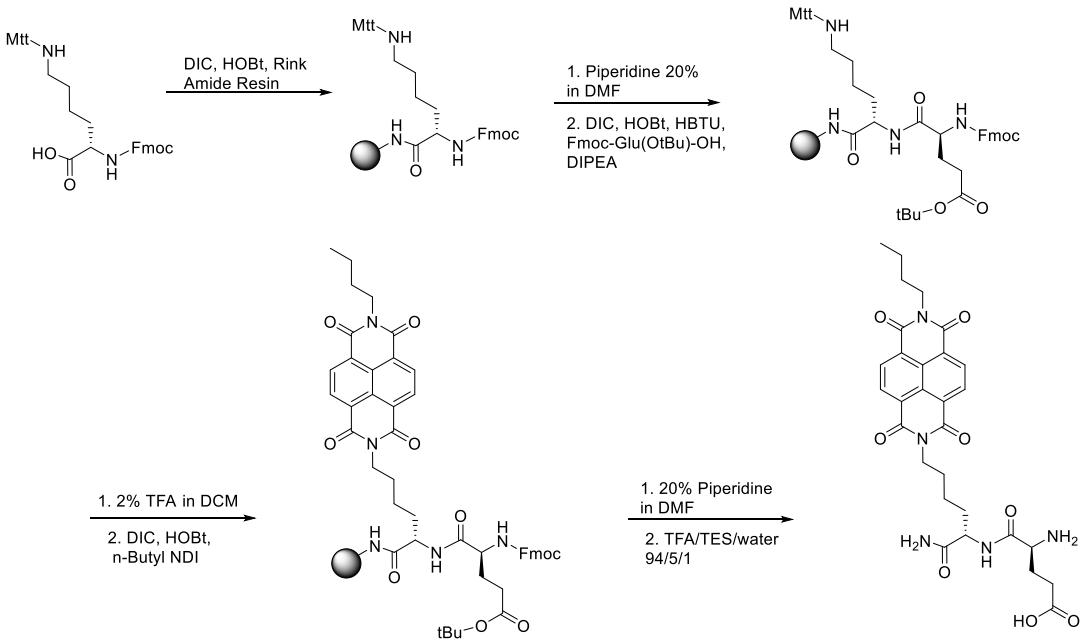


Figure E2. ^1H NMR for EK(NDI). Taken at 400MHz in DMSO.



Scheme E1. The synthetic scheme for the EK(NDI) peptide conjugate. All couplings were made to rink amide resin (0.3 mmol/g loading) in DMF with the reactants listed above.

Transmission Electron Microscopy (TEM). Samples of EK(NDI) aged at around 10 mM were first diluted to 1 mM. Then, the resulting solutions were drop cast onto carbon-coated copper grids (Ted Pella, Inc.) for 1 min. The excess solution was then removed by touching with a Chem-wipe and the grid was stained with 2% (w/w) uranyl acetate solution for 30 s. The excess solution was removed in the same way and the specimen was let dry. The resulting grid was analyzed using the Technai G2 Spirit TEM instrument operating at 80 keV. Images were then analyzed using the Matlab Image Viewer application.

Powder X-ray Difraction (XRD). Samples of EK(NDI) aged at around 10 mM were drop cast onto a silicon wafer. The resulting drop cast wafer was let dry and analyzed by a Bruker D8 Advance X-Ray Powder Diffractometer. The x-ray source was a Cu Kα1 source with a wavelength of 1.54 Å.

Appendix A

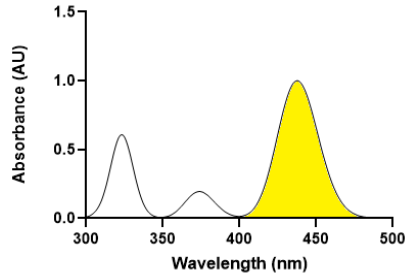
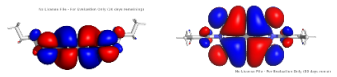
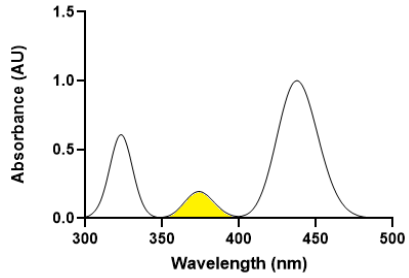
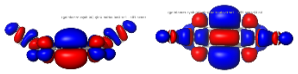
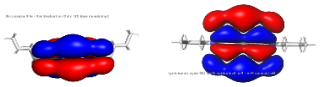
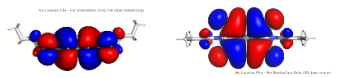
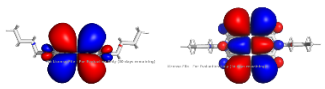
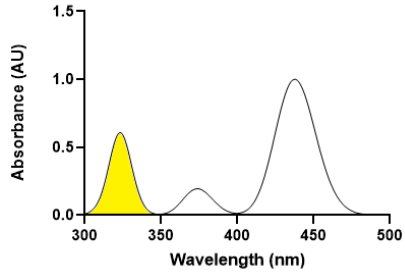
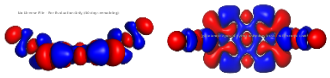
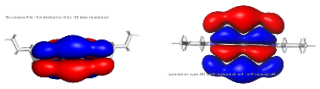
Excitation	Occupied MO	Unoccupied MO
		
		
		
		
		

Table A1. The molecular orbitals paired with their respective electronic transitions, highlighted in yellow on the spectra, as calculated for dibutyl NDI with syn tails with the B3LYP method with a smd water solvent.

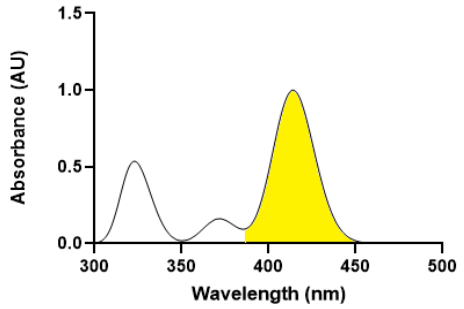
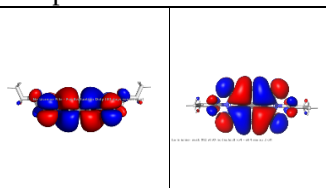
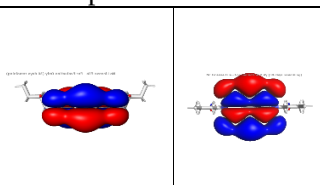
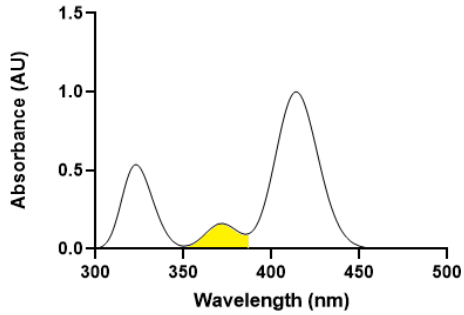
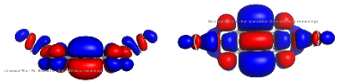
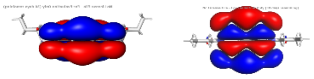
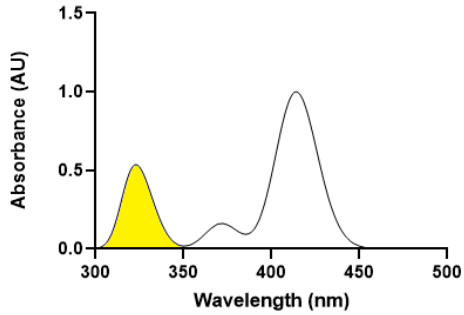
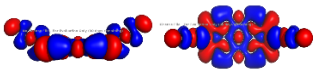
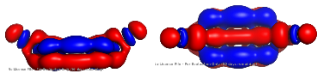
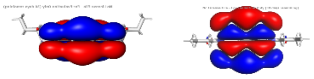
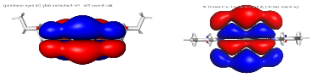
Excitation	Occupied MO	Unoccupied MO
		
		
	 	 

Table A2. The molecular orbitals paired with their respective electronic transitions, highlighted in yellow on the spectra, as calculated for dibutyl NDI with syn tails with the B3LYP method with a smd 2,2,2-trifluoroethanol as solvent.

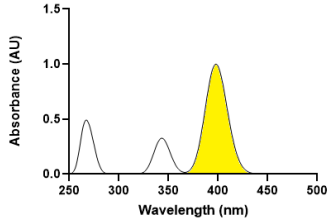
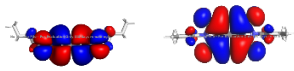
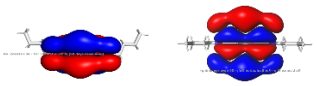
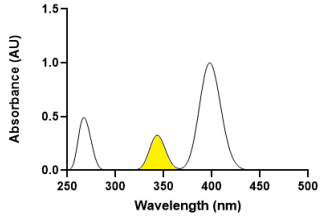
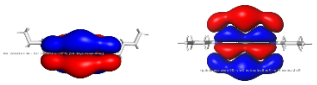
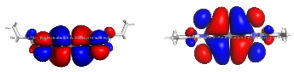
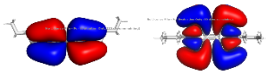
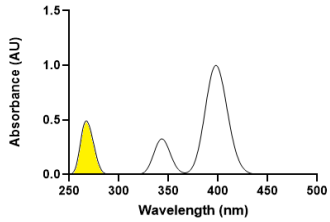
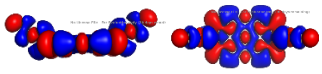
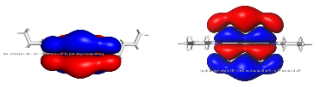
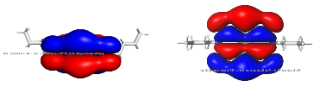
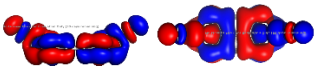
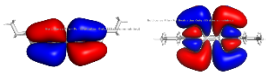
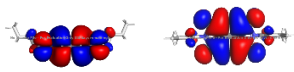
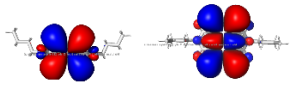
Excitation	Occupied MO	Unoccupied MO
		
		
		
		
		
		
		

Table A3. The molecular orbitals paired with their respective electronic transitions, highlighted in yellow on the spectra, as calculated for dibutyl NDI with syn tails with the CAM-B3LYP method with a smd 2,2,2-trifluoroethanol as solvent.

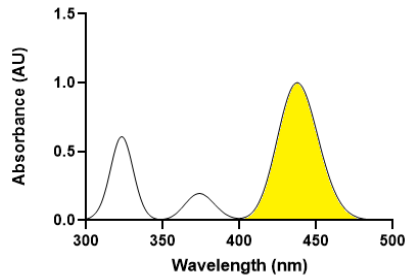
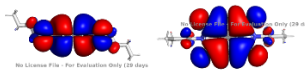
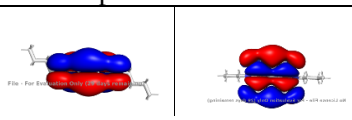
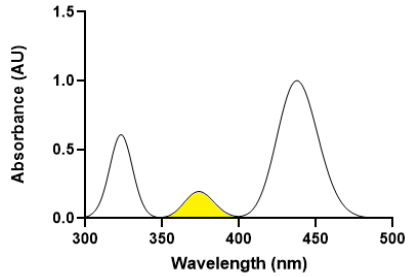
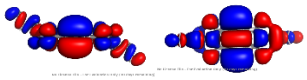
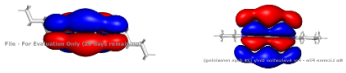
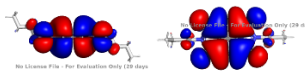
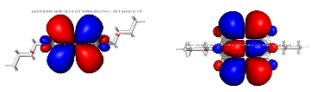
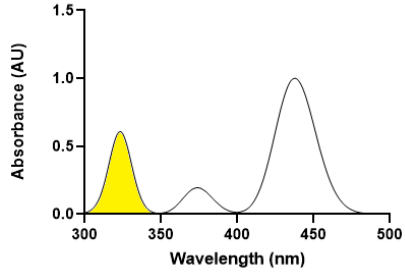
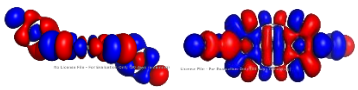
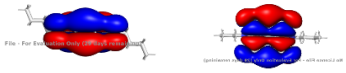
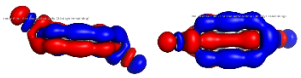
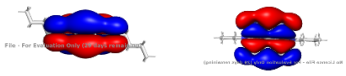
Excitation	Occupied MO	Unoccupied MO
		
		
		
		
		

Table A4. The molecular orbitals paired with their respective electronic transitions, highlighted in yellow on the spectra, as calculated for dibutyl NDI with anti tails with the B3LYP method with a smd water solvent.

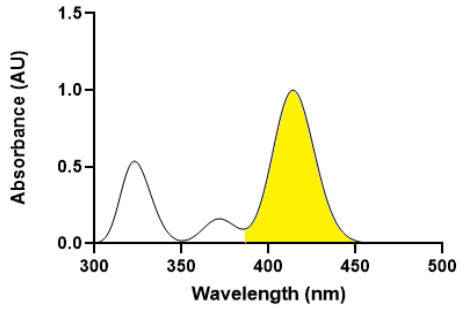
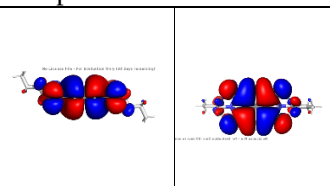
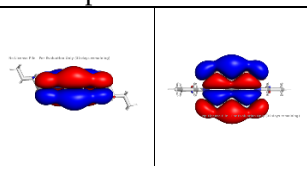
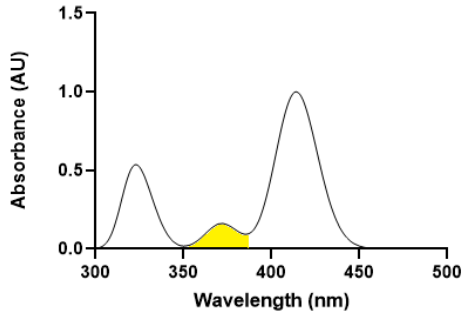
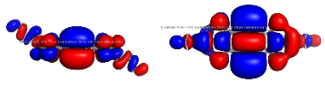
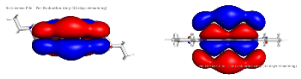
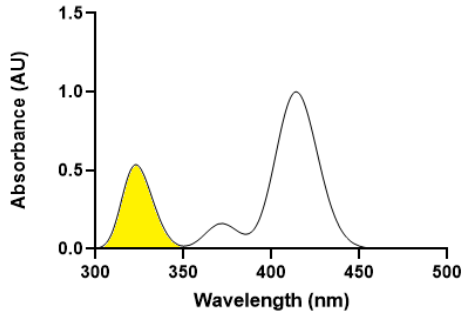
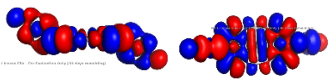
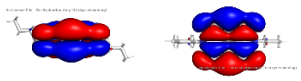
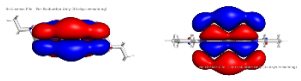
Excitation	Occupied MO	Unoccupied MO
		
		
	 	 

Table A5. The molecular orbitals paired with their respective electronic transitions, highlighted in yellow on the spectra, as calculated for dibutyl NDI with anti tails with the B3LYP method with a smd 2,2,2-trifluoroethanol as solvent.

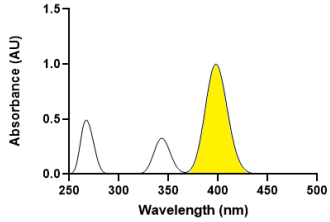
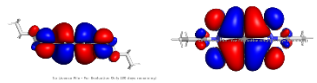
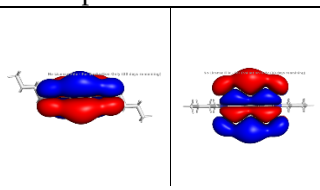
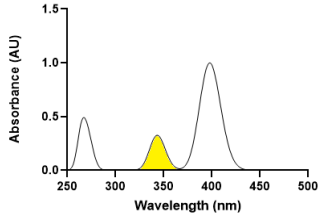
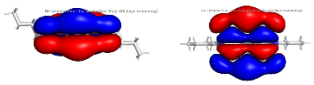
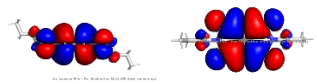
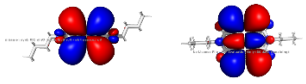
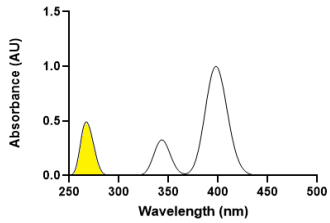
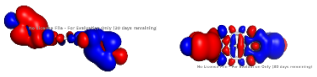
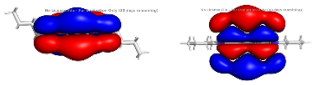
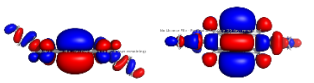
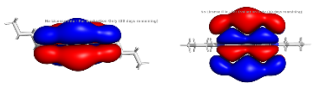
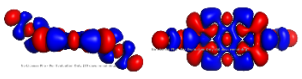
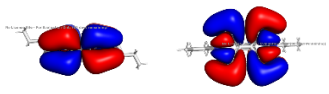
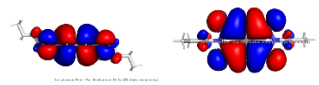
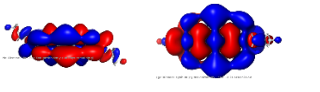
Excitation	Occupied MO	Unoccupied MO
		
		
		
		
		
		
		

Table A6. The molecular orbitals paired with their respective electronic transitions, highlighted in yellow on the spectra, as calculated for dibutyl NDI with anti tails with the CAM-B3LYP method with a smd 2,2,2-trifluoroethanol as solvent.

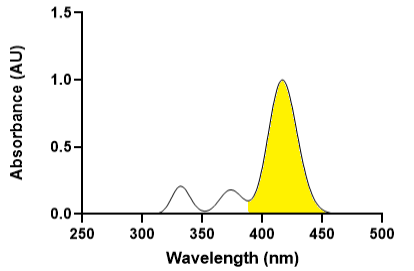
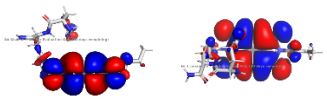
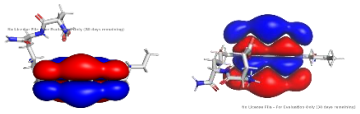
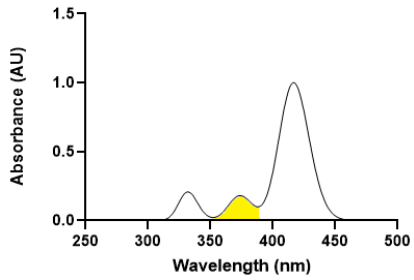
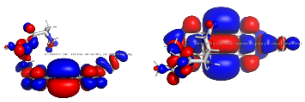
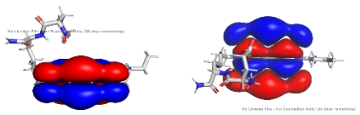
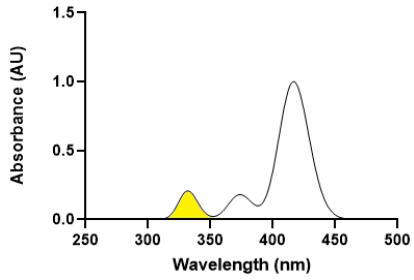
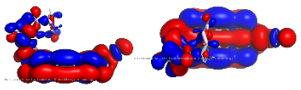
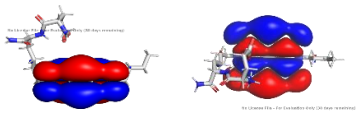
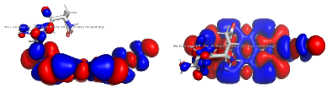
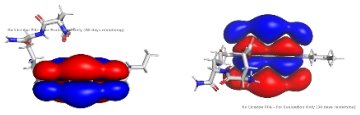
Excitation	Occupied MO	Unoccupied MO
		
		
		
		

Table A7. The molecular orbitals paired with their respective electronic transitions, highlighted in yellow on the spectra, as calculated for EK(NDI) with the B3LYP method and a smd solvation in water.

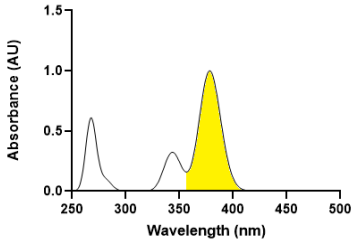
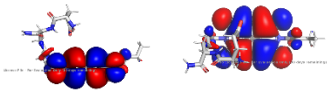
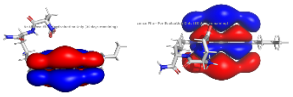
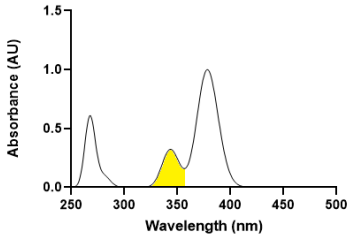
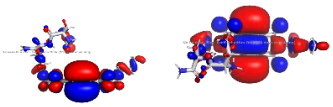
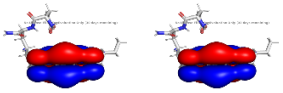
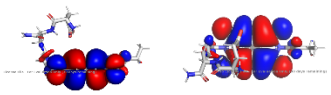
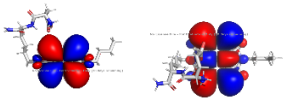
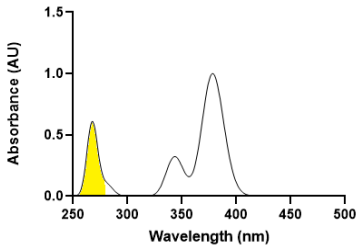
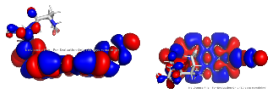
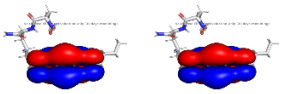
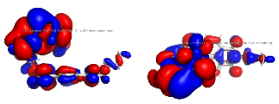
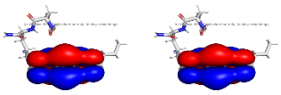
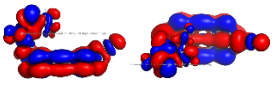
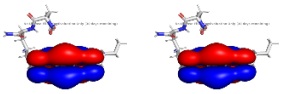
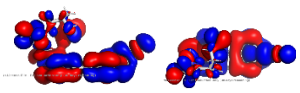
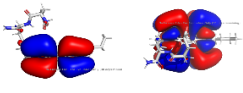
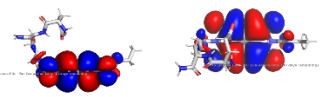
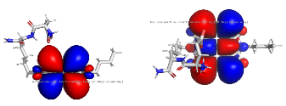
Excitation	Occupied MO	Unoccupied MO
		
		
		
		
		
		
		
		

Table A8. The molecular orbitals paired with their respective electronic transitions, highlighted in yellow on the spectra, as calculated for EK(NDI) with the CAM-B3LYP method with smd solvation in water.

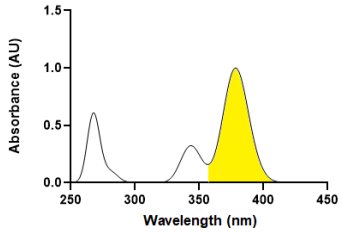
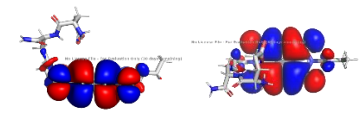
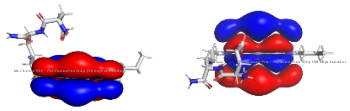
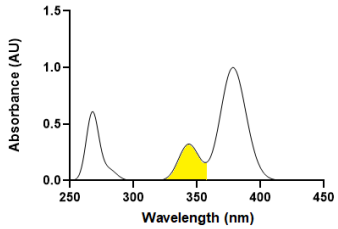
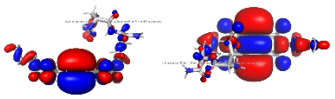
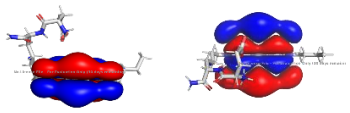
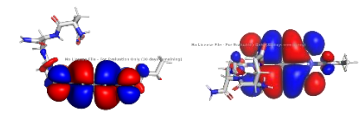
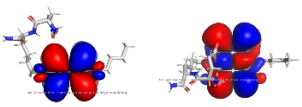
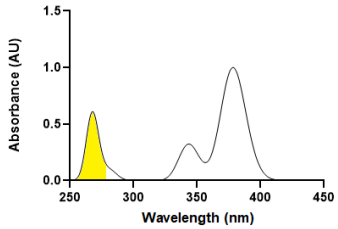
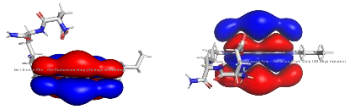
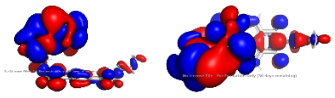
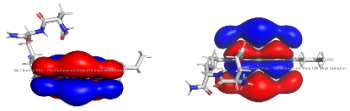
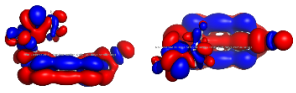
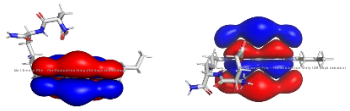
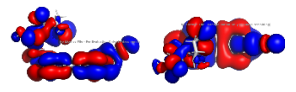
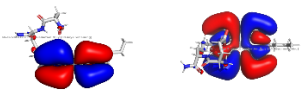
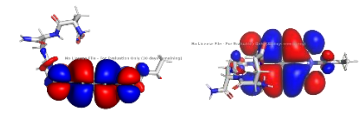
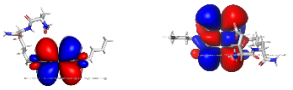
Excitation	Occupied MO	Unoccupied MO
		
		
		
		
		
		
		
		

Table A9. The molecular orbitals paired with their respective electronic transitions, highlighted in yellow on the spectra, as calculated for EK(NDI) with the CAM-B3LYP method with smd solvation in 2,2,2-trifluoroethanol.

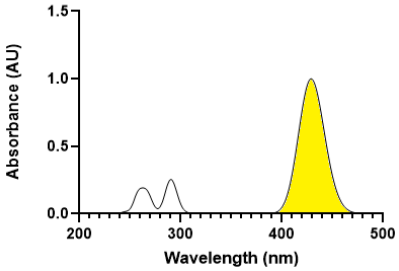
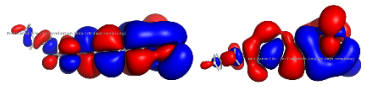
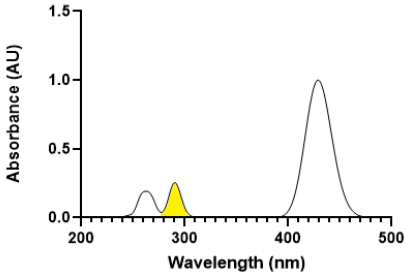
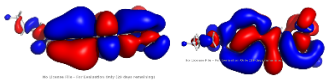
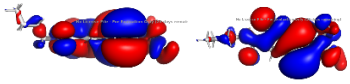
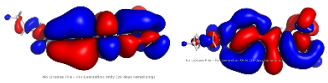
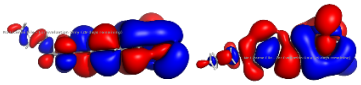
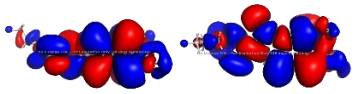
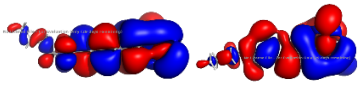
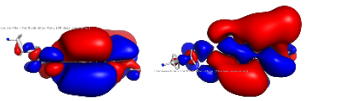
Excitation	Occupied MO	Unoccupied MO
		
		
		
		
		

Table A10. The molecular orbitals paired with their respective electronic transitions, highlighted in yellow on the spectra, as calculated for neutral DAC amide with smd solvation in water.

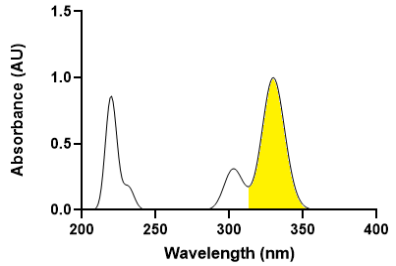
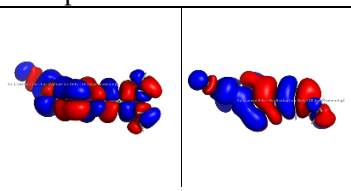
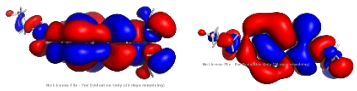
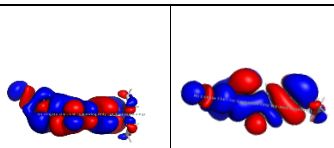
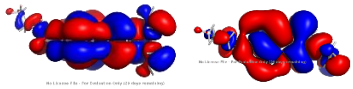
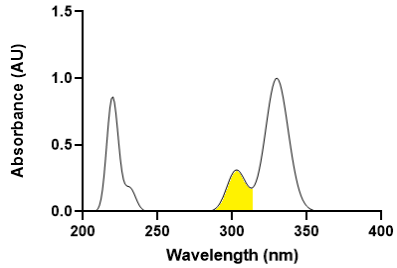
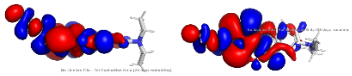
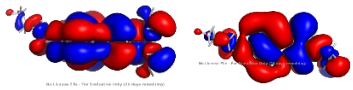
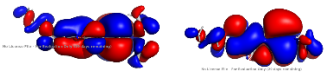
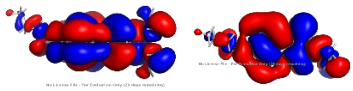
Excitation	Occupied MO	Unoccupied MO
		
		
		
		

Table A10. The molecular orbitals paired with their respective electronic transitions, highlighted in yellow on the spectra, as calculated for protonated DAC amide with smd solvation in water.